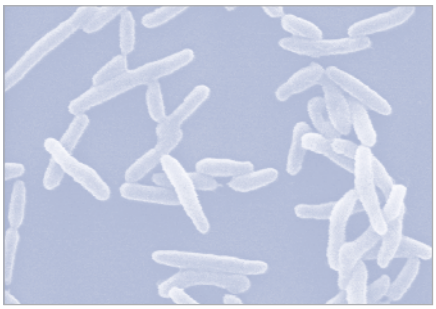
Lactobacillus salivarius Ls-33

DANISCO

CHARACTERISTICS OF THE SPECIES

Lactobacillus salivarius is a Gram-positive, non-spore forming, homofermentative rod and is a common inhabitant of the human intestinal tract and urogenital surfaces (1, 2, 3).

Strains of this species are today widely used in probiotic formulations, both for human and animal application.



SELECTION AND TAXONOMY

Lactobacillus salivarius was described by Rogosa in 1953 as a homofermentative *Lactobacillus* comprising two varieties – *salivarius* and *salicinius* – which then became recognised as sub-species (4). Based on more recent examinations, it has been proposed that *Lactobacillus salivarius* comprises a single species with no intraspecific taxa (5).

L. salivarius Ls-33 has been genetically characterised and properly classified as *L. salivarius* by independent labs using modern genotypic methods including 16S rRNA gene sequence analysis. The strain was originally isolated from an unknown source and has been deposited in the American Type Culture Collection as SD5208.

SAFE FOR CONSUMPTION

Lactic acid bacteria have long been considered safe and suitable for human consumption. Very few instances of infection have been associated with these bacteria, and several published studies have addressed their safety (6-9). Moreover, no *L. salivarius* bacteraemia were identified in a 10-year survey in Finland (10).

L. salivarius is listed in the *Inventory of Microorganisms With Documented History of Use in Human Food* (11). The European Food Safety Authority has also included the species on its Qualified Presumption of Safety list (12).

No acquired antibiotic resistance was detected in *L. salivarius* Ls-33 during screening by the EU-funded PROSAFE project.

The safety of the strain was further evaluated in a colitis mouse model using Trintrobenzenesulphonic acid (TNBS) to induce colitis (13).

In healthy mice, intra-gastric (IG) administration of *L. salivarius* Ls-33 did not show any potential adverse effect on mouse activity, weight, and colon inflammation.

In TNBS-treated mice (mice with very strong colitis), IG administration of *L. salivarius* Ls-33 still resulted in a significant reduction in the inflammatory score compared to scores of the TNBS-positive control group.

High doses (10^{10} CFU) of *L. salivarius* Ls-33 led to no translocation of the organism or abnormal translocation of the intestinal microbiota (13).

GASTROINTESTINAL PERFORMANCE

Resistance to acid and bile

According to the generally accepted definition of a probiotic, the probiotic microorganism should be viable at the time of ingestion to confer a health benefit. Although not explicitly stated, this definition implies that a probiotic should survive GI tract passage and, according to some, colonize the host epithelium.

A variety of traits are believed to be relevant for surviving GI tract passage, the most important of which is tolerance both to the highly acidic conditions present in the stomach and to concentrations of bile salts found in the small intestine.

In vitro studies have shown that *L. salivarius* Ls-33 is very resistant to low pH conditions and survives the presence of bile at concentrations present in the duodenum.

Acid tolerance	++++ (>70% survival in hydrochloric acid and pepsin (1%) at pH 3 for 1h at 37°C)
Bile salt tolerance	++++ (>80% survival in 0.3% bile salt containing medium)
Pepsin resistance	+++ (>40% in 0.3% pepsin containing medium at pH 2 for 1h)
Pancreatin resistance	++++ (>60% survival in 0.1% pancreatin containing medium at pH 8 for 2h)

Selected characteristics of *L. salivarius* Ls-33 (internally generated data):
++++ Excellent; +++ Very good; ++ Good; + Fair

Adhesion to intestinal mucosa

Interaction with the intestinal mucosa is considered important for a number of reasons. Binding to the intestinal mucosa may prolong the time a probiotic strain can reside in the intestine. This interaction with the mucosa brings the probiotic in close contact with the intestinal immune system, giving it a better opportunity to modulate the immune response. It may also protect against enteric pathogens by limiting their ability to colonize the intestine.

Currently, adherence is measured using two *in vitro* cell lines, Caco-2 and HT-29. While this is not a thorough test of the ability of probiotics to adhere to intestinal mucosa in the body, attachment to these cell lines is considered a good indicator of their potential to attach.

L. salivarius Ls-33 has demonstrated excellent adhesion to human epithelial cell lines applied in *in vitro* studies.

Adherence to human intestinal cells *in vitro*	HT-29: **++++** Caco-2: **++++**

Selected characteristics of *L. salivarius* Ls-33 (internally generated data): ++++ Excellent; +++ Very good; ++ Good; + Fair

Inhibition of pathogens

The protective role of probiotic bacteria against gastrointestinal pathogens is highly important to therapeutic modulation of the enteric microbiota. Probiotics are able to inhibit, displace and compete with pathogens, although these abilities are strain-dependent.

The probiotic strains' putative mechanisms of action against pathogenic microorganisms include the production of inhibitory compounds, competition with pathogens for adhesion sites or nutritional sources, inhibition of the production or action of bacterial toxins, ability to coaggregate with pathogens, and the stimulation of immunoglobulin A.

In vitro inhibition is usually investigated using an agar inhibition assay, where soft agar containing the pathogen is laid over colonies of probiotic cultures, causing the development of inhibition zones around the colonies. This effect may be due to the production of acids, hydrogen peroxide, bacteriocins and other substances that act as antibiotic agents as well as competition for nutrients. It should be pointed out, however, that the extrapolation of such results to the *in vivo* situation is not straightforward. The assessment in the table below is based on an *in vitro* assay.

L. salivarius Ls-33 displayed *in vitro* inhibition of selected pathogens.

Pathogen inhibition *in vitro*	*Salmonella typhimurium:* **++** *Staphylococcus aureus:* **++++** *Escherichia coli:* **+++** *Listeria monocytogenes:* **++**

Selected characteristics of *L. salivarius* Ls-33 (internally generated data): ++++ Excellent; +++ Very good; ++ Good; + Fair

The ability to aggregate and coaggregate are desirable properties for probiotics as they are related to the ability to interact closely with pathogens and could avoid or reduce their adhesion to the mucosa. *L. salivarius* Ls-33 showed autoaggregation and coaggregation with several pathogens *in vitro* (14).

L. salivarius Ls-33 also showed the ability to inhibit the adhesion ($P < 0.05$) of *Bacteroides vulgatus* (35.9%), *Clostridium histolyticum* (21.2%), *Clostridium difficile* (40.3%), *Staphylococcus aureus* (37%) and *Enterobacter aerogenes* (36%) *in vitro* (15).

The strain was also able to displace ($P < 0.05$) *B. vulgatus* (58.4%), *C. histolyticum* (22.5%), *C. difficile* (51.9%), *St. aureus* (22.6%), *E. aerogenes* (52.8%) and *L. monocytogenes* (29.2%) *in vitro* (15).

L/D- lactic acid production

Lactic acid is the most important metabolic end product of fermentation processes by lactic acid bacteria and other microorganisms. Lactic acid fermentation has been used for thousands of years for production of fermented foods.

Due to its molecular structure, lactic acid has two optical isomers. One is known as L(+)-lactic acid and the other, its mirror image, is D(-)-lactic acid.

In humans, animals, plants, and microorganisms, L(+)-lactic acid is a normal intermediate or end product of the carbohydrate and amino acid metabolisms. It is important for the generation of energy under anaerobic conditions.

In the organs of humans and animals, the endogenous synthesis of D(-)-lactic acid is very low in quantity. The isomer is normally present in the blood of mammals at nanomolar concentrations and may be formed from methylglyoxal which derives from lipid or amino acid metabolism.

L. salivarius only produces L(+)-lactic acid.

L/D-lactic acid production	**100/0**
Molar ratio	Boehringer Mannheim/ R-Biopharm D-lactic acid/ L-lactic acid UV-method

Internally generated data

IMMUNOMODULATION

An immune system that functions optimally is an important safeguard against infectious and non-infectious diseases. The intestinal microbiota is one of the key elements in the body's immune defence system.

Probiotic bacteria with the ability to modulate certain immune functions may improve the response to oral vaccination, shorten the duration or reduce the risk of certain types of infection, or reduce the risk of or alleviate the symptoms of allergy and other immune-based conditions.

Modulation of the immune system is an area of intense study in relation to the Danisco probiotic range. The goal is to understand how each strain contributes to the maintenance and balance of optimal immune function. The immune system is controlled by compounds known as cytokines. Cytokines are hormone-like proteins made by cells that affect the behaviour of other cells and, thereby, play

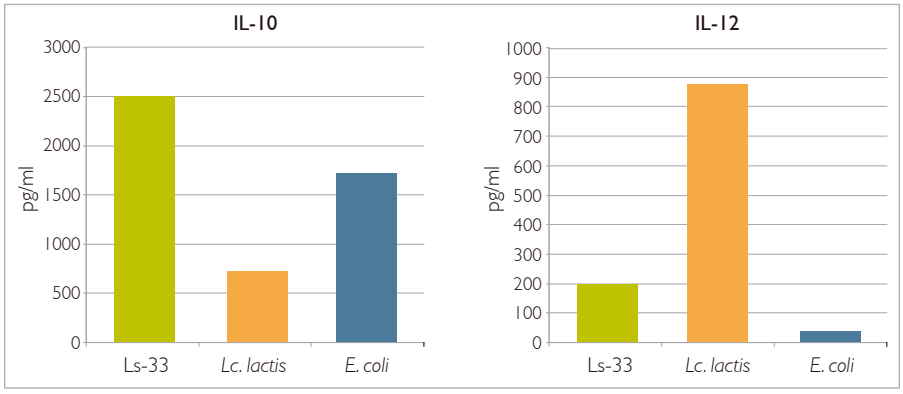


Figure 1. *In vitro* cytokine expression of *L. salivarius* Ls-33 (14).

an important role in the regulation of immune system functions.

In vitro studies

In vitro assays are widely used to define the cytokine profiles of probiotics and, thereby, determine their immunological effects. By measuring the impact of probiotic bacteria during interaction with cytokine-expressing peripheral blood mononucleocytes (PBMCs), information is generated that is useful in determining the ability of each strain to contribute to balanced immune health.

L. salivarius Ls-33 was investigated *in vitro* for its ability to induce the PBMC secretion of selected cytokines: interleukin (IL)-10 and IL-12. The results were compared with *Lactococcus lactis*, a starter culture commonly used in the production of various fermented foods, and *Escherichia coli*, a common member of the intestinal microbiota. IL-10 plays a key role in the control of inflammatory responses to intestinal antigens.

L. salivarius Ls-33 was found to induce IL-10 to a significantly higher degree than *Lc. lactis* and *E. coli*, and IL-12 to a lesser degree, but still higher then *E. coli* (figure 1). This indicates strong anti-inflammatory properties (14).

In vivo animal studies

In line with the results above, *L. salivarius* Ls-33 has further demonstrated an ability to modulate the immune system in an inflammation animal model, validating its ability to contribute to a balanced immune system. The graph below demonstrates the percentage of protection from a chemically-induced intestinal inflammation. *L. salivarius* Ls-33 has led to a highly significant reduction in colitis symptoms and exerts significant protection against intestinal inflammation, demonstrating its ability to interact with and beneficially balance the intestinal mucosal immune response (figure 2) (14).

This result was confirmed in another study using the same model. Here IG administration of *L. salivarius* Ls-33 in TNBS-treated mice resulted in a significant reduction in the inflammatory score but without any significant reduction in weight loss (13).

L. salivarius Ls-33 was further included in a study to investigate the role of dentritic cells (DCs) in the anti-inflammatory potential of probiotic bacteria. DCs belong to the group of antigen-presenting cells (APC) that play a central role in orchestrating immune responses to own and foreign antigens. It has been shown that, after activation with various stimuli, DCs achieve maturation, leading to functional and phenotypic changes.

In this study it was demonstrated that probiotic-treated DCs conferred protection against TNBS-induced colitis in mice, while the administration of untreated DCs did not rescue mice from colitis. In contrast, intra-peritoneal administration of *L. salivarius* Ls-33-treated DCs led to a considerable reduction in the colitis, with reduced weight loss, improved clinical parameters and a significant reduction in macroscopic inflammation scores (figure 3) (15).

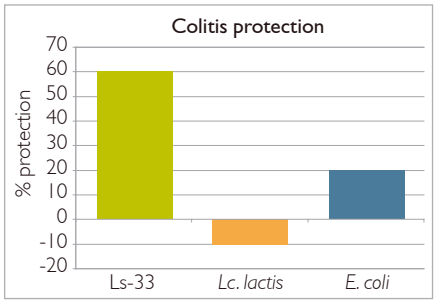


Figure 2. Percentage of protection in an acute murine model of inflammation (TNBS) (14).

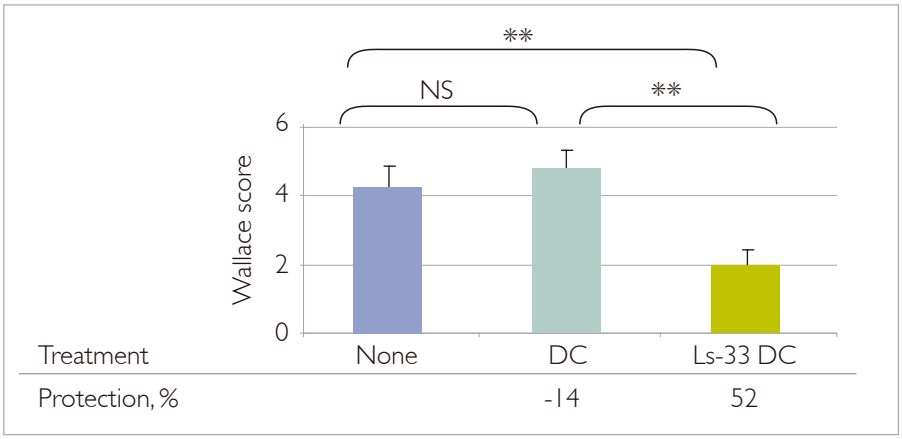


Figure 3. Protective effect of intra-peritoneal administration of LAB-treated BMDCs on acute TNBS-induced colitis in BALB/c mice. Wallace inflammation scores were calculated after a TNBS challenge in mice either not treated (None) or intra-peritoneally-injected with untreated BMDCs (DC) or BMDCs treated with the *L. salivarius* Ls-33 (Ls-33 DC) strain (15).

ANTIBIOTIC RESISTANCE PATTERNS

Antibiotic susceptibility patterns are an important means of demonstrating the potential of an organism to be readily inactivated by the antibiotics used in human therapy.

Antibiotic resistance is a natural property of microorganisms and existed before antibiotics became used by humans. In many cases, resistance is due to the absence of the specific antibiotic target or is a consequence of natural selection.

Antibiotic resistance can be defined as the ability of some bacteria to survive or even grow in the presence of certain substances that usually inhibit or kill other bacteria. This resistance may be:

Inherent or intrinsic: most, if not all, strains of a certain bacterial species are not normally susceptible to a certain antibiotic. The antibiotic has no effect on these cells, being unable to kill or inhibit the bacterium.

Acquired: most strains of a bacterial species are usually susceptible to a given antibiotic. However some strains may be resistant, having adapted to survive antibiotic exposure. Possible explanations for this include:

- A mutation in the gene coding for the antibiotic's target can make an antibiotic less efficient. This type of antibiotic resistance is usually not transferable.
- A resistance gene may have been acquired from a bacterium.

Of the acquired resistances, the latter is of most concern, as it may also be passed on to other (potentially pathogenic) bacteria.

Much concern has arisen in recent years regarding vancomycin resistance, as vancomycin-resistant enterococci are a leading cause of hospital-acquired infections and are refractory to treatment. The transmissible nature of genetic elements that encode vancomycin resistance in these enterococci is an important mechanism of pathogenicity.

Resistance to vancomycin in certain lactobacilli, including *L. salivarius*, pediococci and leuconostoc is due to intrinsic factors related to the composition of their cell wall, and not due to any transmissible elements (16). *L. salivarius* Ls-33 has been confirmed through PCR testing to be free of *Enterococcus*-like vancomycin-resistance genes.

As of today no case of antibiotic resistance transfer has ever been identified and reported for lactic acid bacteria used in foods and feed.

The antibiotic susceptibility patterns for *L. salivarius* Ls-33 are summarised in table 1.

BENEFIT SUMMARY

Extensive *in vitro* and *in vivo* studies support the health-enhancing, probiotic properties of *L. plantarum* Lp-115. Following is a summary of these attributes:

- Long history of safe use
- Well-suited for intestinal survival
  - High tolerance to gastrointestinal conditions (acid, bile, pepsin and pancreatin)
  - Strong adhesion to intestinal cell lines
- Ability to inhibit common pathogens
- Beneficial modulation of immune functions
  - May influence immune regulation, as demonstrated by increased induction of IL-10 *in vitro*
  - *L. salivarius* Ls-33 has shown anti-inflammatory properties, as demonstrated by significant protection against colitis in an animal model

***Lactobacillus salivarius* Ls-33 antibiogram**	
Amoxicillin	S
Ampicillin	S
Ceftazidime	R
Chloramphenicol	R
Ciprofloxacin	R
Clindamycin	S
Cloxacillin	R
Dicloxacillin	R
Erythromycin	I
Gentamicin	R
Imipenem	R
Kanamycin	R
Neomycin	R
Nitrofurantoin	R
Penicillin G	S
Polymixin B	R
Rifampicin	R
Streptomycin	R
Sulfamethoxazole	R
Tetracycline	R
Trimethoprim	R
Vancomycin	R

S = Susceptible (minimum inhibitory concentration ≤ 4μg/ml)
I = Intermediate (minimum inhibitory concentration = 8 to 32μg/ml)
R = Resistant (minimum inhibitory concentration ≥ 64μg/ml)

Table 1.

REFERENCES

Publications on *L. salivarius* Ls-33 in bold.

1. Kandler, O. & Weiss, N. (1986). Genus *Lactobacillus*, p. 1209-1234. In Sneath, P.H.A., Mair, N.S., Sharpe, M.E. & Holt, J.G. (ed.). Bergey's manual of systematic bacteriology, vol. 2. Williams and Wilkins, Baltimore, Maryland.

2. Mitsuoka, T. (1996). Intestinal flora and human health. Asia Pacific J. Clin. Nutr. 5:2-9.

3. Reuter, G. (2001). The *Lactobacillus* and *Bifidobacterium* microflora of the human intestine: composition and succession. Curr. Issues Intest. Microbiol. 2:43-53.

4. Rogosa, M., Wiemann, R.F., Mitchell, J.A., Disraely, M.N. & Beaman, A.J. (1953). Species differentiation of oral lactobacilli from man including description of *Lactobacillus salivarius* nov spec and *Lactobacillus cellobiosus* nov spec. Journal of Bacteriology. 65(6):681-99.

5. Yin Li, Raftis, E., Canchaya, C., Fitzgerald, G. F., Van Sinderen, D. & O'Toole, P. W. (2006). Polyphasic analysis indicates that *Lactobacillus salivarius* subsp. *salivarius* and *Lactobacillus salivarius* subsp. *salicinius* do not merit separate subspecies status. International Journal of Systematic and Evolutionary Microbiology. 56 (10), 2397-2403.

6. Aguirre, M. & Collins, M.D. (1993). Lactic acid bacteria and human clinical infections. J. Appl. Bact. 75:95-107.
7. Gasser, F. (1994). Safety of lactic acid bacteria and their occurrence in human clinical infections. Bull. Inst. Pasteur. 92:45-67.
8. Salminen S., von Wright, A., Morelli, L., Marteau, P., Brassart, D., de Vos, W.M., Fonden, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S.-E. & Mattila-Sandholm, T. 1998. Demonstration of safety of probiotics – a review. Int. J. Food Prot. 44:93-106.
9. Borriello, S.P., Hammes, W.P., Holzapfel, W., Marteau, P., Schrezenmeir, J., Vaara, M. & Valtonen, V. (2003). Safety of probiotics that contain lactobacilli or bifidobacteria. Clin. Infect. Dis. 36:775-780.
10. Salminen, M.K., Tynkkynen, S., Rautelin, H., Saxelin, M., Vaara, M., Ruutu, P., Seppo Sarna, S., Valtonen, V. & Järvinen, A. (2002). *Lactobacillus* Bacteremia during a Rapid Increase in Probiotic Use of *Lactobacillus rhamnosus* GG in Finland. Clinical Infectious Diseases. 2002. 35:1155-60.
11. Mogensen, G., Salminen, S., O'Brien, J., Ouwehand, A.C., Holzapfel, W., Shortt, C., Fonden, R., Miller, G.D., Donohue, D., Playne, M., Crittenden, R., Salvadori, B. & Zink, R. (2002). Inventory of microorganisms with a documented history of safe use in food. Bulletin of the International Dairy Federation. 377:10-19.
12. List of taxonomic units proposed for QPS status http://www.efsa.europa.eu/EFSA/Scientific_Opinion/sc_op_ej587_qps_en.pdf.
13. Daniel, C., Poiret, S., Goudercourt, D., Dennin, V., Leyer, G. & Pot, B. (2006). Selecting Lactic Acid Bacteria for Their Safety and Functionality by Use of a Mouse Colitis Model. Applied and Environmental Microbiology. 72(9):5799-5805.
14. Collado, M.C., Meriluoto, J. & Salminen, S. (2007). Adhesion and aggregation properties of probiotic and pathogen strains. Eur Food Res Technol. DOI 10.1007/s00217-007-0632-x.
15. Collado, M.C., Meriluoto, J. & Salminen, S. (2007). Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Letters in Applied Microbiology. doi:10.1111/j.1472-765X.2007.02212.x.
16. Foligne, B., Nutten, S., Grangette, C., Dennin, V., Goudercourt, D., Poiret, S., Dewulf, J., Brassart, D., Mercenier, A. & Pot, B. (2007). Correlation between *in vitro* and *in vivo* immunomodulatory properties of lactic acid bacteria. World Journal of Gastroenterology. 13(2):236-243.
17. Foligne, B., Zoumpopoulou, G., Dewulf, J., Ben Younes, A., Chareyre, F., *et al.* (2007). A Key Role of Dendritic Cells in Probiotic Functionality. PloS ONE 2(3): e313. doi:10.1371/journal.pone.0000313.
18. Delcour, J., Ferain, T., Deghorain, M., Palumbo, E., Hols, P. (1999). The biosynthesis and functionality of the cell-wall of lactic acid bacteria. Antonie Van Leeuwenhoek. 76(1-4):159-84.

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